

(NASA-CR-196004) [EFFECT OF
MICROGRAVITY ON PLANT GROWTH] Final
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Original Research Goals: The overall goal of my research is to determine the effect of microgravity proper on plant growth (metabolism and cell wall formation). In addressing this goal, the work conducted during this granting period was divided into three components: (1) Analyses of various plant tissues previously grown in space aboard MIR Space Station; (2) Analyses of wheat tissues grown on Shuttle flight STS-51; and (3) Phenylpropanoid metabolism and plant cell wall synthesis (earth-based investigations).

Background: A number of studies (mainly conducted by former Soviet-bloc scientists) reported preliminary data that apparently demonstrated various striking effects of the space-flight environment on plant growth and development, particularly with respect to cell wall formation (summarized in Halstead and Dutcher, 1984, 1987; Dutcher *et al.*, 1994 and references therein). For example, in studies ranging from unicellular algae to angiosperms, it has been reported that substantive differences in growth and development occur in space when compared with their 1g controls. These differences purportedly include smaller plants, alterations in endoplasmic reticula and ribosomes, "swollen" mitochondria, changes in morphology of the cisternae of dictyosomes, random distribution of amyloplasts (with smaller starch grains), multiple nuclei, chromosomal aberrations, reduction or (partial) inhibition of cell mitosis, disturbances in the spindle mechanism, differences in cell size and shape, diminution of cellular aggregation capability, alteration of rate(s) of differentiation presumed to lead to more rapid aging, thinner cell walls (with apparently altered biopolymer composition and architecture), disoriented roots (growing upwards rather than downwards), and substantial differences in essential element composition (see Halstead and Dutcher, 1984, 1987; Krikorian and Levine, 1991; Dutcher *et al.*, 1994 and references therein). At first glance, the absence of gravity can be interpreted as having a profound and fundamentally important effect on normal growth and development.

Research Findings

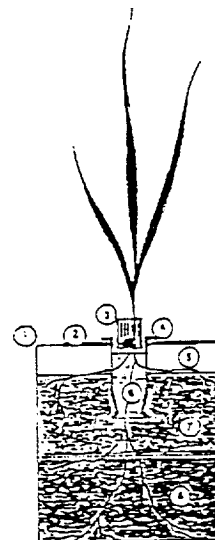
1. **Analyses of Tissues Previously Grown aboard MIR Space Station.** In collaboration with Russian scientists, Drs. G. Neichitailo and A. Mashinsky, tissues from various space-grown plants were provided for detailed cytological and chemical/biochemical characterization. These included wheat plants (*T. aestivum* L.), radish (*R. sativa*), Chinese cabbage (*B. pekinensis*), and the woody plant hybrid "limoniya". The *T. aestivum* plantlets were grown in space for 14 to 21 days on two separate missions, and were chemically fixed on return to earth in preparation for cytological analyses. In contrast, the tissues of radish and cabbage were freeze-dried upon recovery, whereas the limoniya hybrids were recovered as dead woody plant material following missions lasting one and two years duration, respectively. All of the *T. aestivum* plants were grown in a Svetobloc apparatus. However, the tissues provided for detailed cytological and cell-wall architecture studies were so artifact prone (both flight and control plants), that no convincing scientific data in support of previous Soviet-bloc claims could be made. The same held true for the limoniya plants, although it did appear that the cell walls were qualitatively similar to their earth-grown counterparts. In the next stage of our analyses (using radish and Chinese cabbage tissues grown in Svet greenhouses), significant differences were observed between space and earth-grown plant materials. Using a combination of solid-state carbon-13 NMR spectroscopy, acetyl bromide, and thioacidolytic lignin determinations, it was tentatively concluded that the *R. sativa* plants produced more pectinaceous noncellulosic polysaccharide polymers, with cell walls having a lower α -cellulose content and a lignin of greatly reduced sinapyl alcohol moieties (Lewis, 1993). However, it was subsequently disclosed by the Russian investigators that these space-grown plants only reached ca 1/10 the size/fresh weight of their ground control counterparts. This deleterious effect on growth (and the viability of the space-grown plants themselves) was subsequently found to be, in large part, due to poor fluid distribution properties in microgravity in the balkanite (soil-like particulate matter) in the Svet greenhouse, i.e., different "wetting properties" were experienced in microgravity which adversely affected the ability of water and nutrients to effectively reach the required root zones. This occurs because of altered physicochemical effects in microgravity which affect surface tension and molecular attraction forces and, hence, both fluid/nutrient delivery and uptake. Fluids, instead, tend to deposit in unexpected or undesirable ways on plant surfaces and growth substrates, which can also significantly impede either uptake or air movement. Taken together, these differences noted in metabolism, etc., are presumed to be largely due to the stresses accompanying poor nutrient and water delivery to the growing plants. It should, therefore, be evident that these results put into question the significance of many of the previous plant space-flight studies.

2. **Growth of Wheat (*T. aestivum* L.) Plants on Space Shuttle Mission STS-51.** It, therefore, follows that if we were to convincingly establish the effects of microgravity, from either a basic science perspective or for more practical biomass growth applications, these undesirable interactions between micro-g, other physical environmental parameters, and plant responses needed to be fully recognized, addressed, and their effects pragmatically minimized. [These concerns cannot be trivialized since they, if not corrected, would ultimately result in the inability to conduct reliable research on Space Station Alpha, and the compromise of the entire scientific enterprise on the space biology of plants.] In order to circumvent some of the most difficult and immediate problems in growing plants in space, we developed, flight-tested, and compared two distinct root module support systems suitable for use in the U.S. Plant Growth Unit (PGU). This was viewed as an essential first step in eliminating or minimizing some of the

more severe difficulties alluded to above. Both modules were designed to obviate the need for sporadic replenishment of a water/nutrient supply for the duration of a typical shuttle mission, i.e., for 10 to 12 days. The first was based on a "nutrient pack" concept, containing a modified gel with mineral nutrients and encapsulated water (Fig. 1). It was developed in order to grow wheat from seed to maturity (Heyenga *et al.*, in preparation) without addition of nutrients or water during the growth cycle. The second was based upon a "horticulture foam" which had been adapted for space use in the PGU by Krikorian (cf. Levine and Krikorian, 1992a), specifically for aseptically recovering in-space generated roots from tissue culture-generated propagules for chromosomal studies (Krikorian *et al.*, 1992). Both root modules were incorporated into a PGU as part of the recent STS-51 mission which returned from orbit following a 10-day mission. Because of difficulties in installing an air exchange system, the experiment was conducted in sealed chambers.

Figure 1: WSU "Nutrient Pack"

1. Polypropylene envelope
2. Gas diffusion membrane
3. Seed support matrix
4. Support column
5. Air phase
6. Germination medium
7. Basic nutrient medium
8. Enriched medium



Using this approach, it was found that the seedlings in the "nutrient pack" grew very well in space with little difference observed in shoot and root tissue growth/fresh weight over the 10-day flight when compared to the corresponding growth controls. Visual examination (and appropriate measurements of height/fresh weight) also revealed that the plants grew well on the horticulture foam. But the foam seedlings differed from their "nutrient pack" analogues in terms of degree of root branching and a tendency of some roots to emerge from the foam in space since they are only loosely restrained in the foam system, whereas they are fully entrapped in the "nutrient pack". Upon recovery at Kennedy Space Center, seedling tissues were either immediately fixed or frozen in preparation for more comprehensive analyses. (Our preference to have this done in orbit could not be met for that particular mission.) Both sets of plants (space and ground-based 1g controls) have since been subjected to intense scrutiny. For example, electron microscopic examination of cross-sections of serial sections of wheat shoots and roots revealed essentially no differences. That is, the organelles in the cytosol looked to be very similar, and in the root cell walls there were no measurable changes in the cellulose microfibril orientation/cell wall thickness as revealed by freeze fracture/TEM/SEM analyses (He *et al.*, in preparation). This latter observation is particularly important since it strongly implies that either the process guiding microfibril orientation is fully sensitive to even a microgravity stimulus, or that it occurs independent of the g-force experienced. In summary, this is the first data set that we are aware of that has been obtained whereby excellent growth, *relative to the 1g controls*, was attained in space over a relatively "long duration" (cf. Levine and Krikorian, 1992b). Importantly, cell wall assembly was not apparently significantly affected. These results are in direct contradiction to other studies, whose plants (as indicated earlier) may have, it can now be hypothesized, suffered from various stresses in microgravity such as perturbations in water and nutrient relations during growth in space. It must be emphasized, however, that our results represent a single, carefully executed experiment, but whose findings need to be confirmed by a reflight. Other more detailed chemical and biochemical analyses are currently underway and should reveal the extent of any metabolic differences encountered during space-flight.

3. Phenylpropanoid Metabolism and Plant Cell Wall Synthesis. Of the biochemical processes occurring within vascular plants, the phenylpropanoid pathway (leading to lignans, neolignans, and lignins), and its merging with the acetate pathway to give suberins, is arguably the most important for the successful land colonization by plants (Lewis and Davin, 1994). This is because it affords the means whereby plants can engender the ability to withstand compressive forces (e.g., to counteract gravity) and to conduct water and nutrients (via lignin synthesis), as well as forming water diffusion resistance barriers (i.e., suberin layers). Thus, variations in plant cell wall chemistry (particularly phenylpropanoid metabolic changes) have enabled plants to effectively evolve to counteract gravitational forces as well as provide facile mechanisms for correctional responses to various gravitational stimuli, e.g., in reaction wood formation. A principal focus of our current research is, therefore, to precisely and systematically dissect the essentially unknown mechanism(s) of vascular plant cell wall assembly, particularly with

respect to its phenolic constituents, i.e., lignin and suberin formation, and how gravity impacts upon these processes. The progress to this comprehensive goal (during this funding period) is as follows:

a. **Lignin Synthesis and Cell Wall Assembly.** Our most recent research endeavors have addressed development of a model system to investigate early stages of lignin/secondary cell wall formation, the delineation of the sequential steps involved in lignin assembly, and regulation of the pathway itself. The following has been discovered: using cell cultures of *Pinus taeda* (Eberhardt *et al.*, 1993), it has been possible to attain a cell line capable of undergoing a developmental-like transition from an unlignified primary wall to a lignified secondary wall (~S₁ deposition). Judicious carbon-13 labelling established that the lignin so formed was a relatively high fidelity copy of a softwood gymnosperm lignin. We have also discovered that lignin synthesis in these cell cultures can be totally inhibited using H₂O₂ scavengers (e.g., KI) (Nose *et al.*, 1994). In such instances, only the monolignols, *p*-coumaryl, and coniferyl alcohols were formed, but without lignin synthesis itself occurring. During lignification/secondary wall synthesis, it was also observed that *p*-coumaryl and coniferyl alcohols had different metabolic fates prior to polymerization (Bernards *et al.*, 1994a). Thus, coniferyl alcohol underwent facile dimer formation prior to lignin synthesis proper, whereas *p*-coumaryl alcohol did not react until polymerization was initiated. Additionally, using *Forsythia* species, we have shown that three types of phenol-coupling enzymes are present. The most remarkable is a hitherto uncharacterized stereoselective oxidase which catalyzes the coupling of two *E*-coniferyl alcohols to give (+)-pinoresinol (Paré *et al.*, 1994); this is being purified to apparent homogeneity. The other coupling enzymes are O₂-requiring laccase(s) and H₂O₂-dependent peroxidase(s), respectively, both of which afford racemic products. The significance of these findings is as follows: lignins (composition and content) vary with cell type and species, and gravitational corrections of plants also result in altered lignin composition in the tissues so affected (e.g., a higher *p*-coumaryl alcohol content in reaction wood). Our recent findings now provide a means to systematically define how these compositional changes are regulated, i.e., what factors differentially control individual monomeric synthesis and how precise mechanisms of assembly are controlled in different cell types. A detailed understanding of such processes is essential if we are to define how lignin synthesis is induced and regulated during "normal" and "gravity correctional" responses.

b. **Suberization and Cell Wall Synthesis.** Nothing is yet known about how gravity affects the suberin forming process. In the very first stages of addressing this question, we have first investigated the chemical nature of the aromatic domain of suberin. Using a combination of carbon-13 labelling techniques, *in situ* solid state ¹³C-NMR spectroscopic analysis, and comparison with selected model compounds, however, we have found that the phenolic domain of suberin is comprised almost exclusively of an unprecedented, covalently-linked hydroxycinnamate polymer matrix (Bernards *et al.*, 1994b) and not lignin, as originally proposed by others. The next phase of our studies is to establish how gravity impacts upon this process.

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